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DIFFERENTIATION OF CATALATIC ACTIVITY OF *SACCHAROMYCES CEREVISIAE* CYTOCHROME B_2: L-LACTATE DEHYDROGENASE FROM CATALASE T BY STARCH GEL ELECTROPHORESIS (U)

by

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ABSTRACT

Crude extracts of yeast exhibit two catalase activity bands on starch gel zymograms. Antibody prepared against catalase T, specifically precipitated fast moving catalatic band of catalase T, but did not affect the slow moving catalatic band due to cytochrome b_2.

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INTRODUCTION

Some years ago, in the course of purifying a highly active and typical catalase (catalase T) in S. cerevisiae (22), we became aware of the presence, in the same crude extracts, of three other proteins which also possessed catalatic activity; two of these were then purified to homogeniety (23). One of these was an atypical catalase which we called catalase-A (24), while the second one, containing non-heme iron and RNA, we

called the catalatic nucleoprotein (25). Purification and properties of these two proteins have been described (24, 25). Ruis and his associates have elucidated the biosynthesis of catalase T and catalase A in yeast by means of double labelling with L-[^{3}H]-leucine and ^{59}Fe coupled with immunoprecipitation techniques (29). More recently, they have studied the regulation of these hemoproteins in yeast (8) and have isolated and cloned the gene for catalase T (27). This communication presents evidence to show that the fourth protein in the yeast crude extract is the L-lactate: ferricytochrome C oxidoreductase (EC 1.1.2.3) which is commonly referred to as the yeast cytochrome b_2 (1).

Catalase (H_2O_2:H_2O_2 oxidoreductase EC 1.11.1.6) catalyzes the decomposition of hydrogen peroxide directly into molecular oxygen and water. This mode of reaction distinguishes it from peroxidase (donor: H_2O_2 oxidoreductase EC 1.11.1.7) which can also utilize H_2O_2 as substrate (12). The substrate specificity of catalase was made use of by Scandalios (18) to localize, and to identify the presence of catalase(s) on starch gel zymograms; this method has been widely used. While non-enzymatic destruction of H_2O_2 by ferric salts and by a number of haemoproteins has long been known (15), the possibility that the very weak catalatic activity of these metallic salts and proteins might give a positive reaction that might be misinterpreted for the presence of catalase on the zymogram has not been considered. There is now a voluminous literature (2, 5-7, 9, 13, 14, 18-21) dealing with catalase isoenzyme demonstrated by means of starch gel zymograms of crude extracts of a variety of species. We show in this communication that one of the band in a "catalase" zymogram of yeast crude extracts is due to the L-lactacte:ferricytochrome C oxidoreductase (EC 1.1.2.3) which is commonly referred to as yeast cytochrome b_2(1). Workers in this field should be aware of this, in order to avoid possible misinterpretation of their data.

MATERIALS AND METHODS

Yeast cytochrome b_2, L-lactate dehydrogenase (LDH), was purchased from Worthington (Freehold, New Jersey) and yeast catalases were purified from commercial yeast (Standard Brands) as described (1, 3, 4). Antibody to purified catalase T was prepared by subcutaneously injecting, in the thigh, two 2-3 month old male rabbits with 1.0 mL of an enzyme preparation containing 4.0 mL of pure antigen (approximately 3.0 mg protein) and an equal volume of Freund's (complete) adjuvant. Three injections were given to each rabbit at intervals of one week and blood was collected from the ear at 3 to 8 days after the last injection. The γ-globulin fraction was precipitated from pooled sera by the method of Keckwick (11), resuspended in phosphate buffer (5×10^{-3} M, pH 7.0) and dialyzed for 48 h at 4°C against the same buffer. The resulting preparation was used as the anti-catalase T serum.

The titanium sulfate method for catalase activity is expressed as the pseudo-first order rate constant per second per mg protein (22). Yeast cytochrome b_2 activity was assayed by the method of Appleby and Morton (1) and specific activity is expressed as units per mg protein where one unit of activity is equal to the reduction of one micromole of ferricyanide per minute at 25°C. In some experiments, the catalatic activity of cytochrome b_2 was assayed in the presence of 1×10^{-5} to 1×10^{-1} M 3-amino-1,2,4-triazole (AT). A modified method of Heim, Appleman, and Pyfrom (4) for catalase inhibition *in vitro* was employed. The method was as follows; one mL portions of enzyme solution containing a final concentration of 5×10^{-3} M phosphate buffer (pH 7.0), 1×10^{-3} M ethylenediaminetetraacetic acid (EDTA), 5 mg enzyme and a given concentration of AT, in the absence of H_2O_2, were incubated at 37°C for ½ hour after which the residual catalase and LDH activities were determined. Starch gel electrophoresis was carried out as previously described (3). Electrophoresis was performed at 4°C for

4 to 5 hours at 200 volts. The gel was sliced and the catalase activity was localized by a modification of the method of Scandalios (18). The sliced gels were incubated at room temperature in 1 x 10^{-1} M H_2O_2 in 5 x 10^{-3} M phosphate buffer (pH 7.0). After 10 min, gels were rinsed thoroughly with distilled water and treated for 10 to 15 sec with a solution of 15% potassium iodide (KI) prepared in 5% acetic acid. The KI solution was poured off as soon as the background of the gels turned pale blue and the gels were rinsed thoroughly in a continuous stream of tap-water. The gels appeared dark blue except for the clear band where catalase activity was localized. The stained gels were preserved in a solution of ethanol, glycerol, and water (v/v 5:1:5). Protein concentration was determined by Lowry's method (17) with bovine serum albumin as standard.

RESULTS AND DICUSSIONS

The cytochrome b_2 used in the present study was found to be homogeneous by analytical ultracentrifugation. By our method of assay, its catalatic activity was approximately 0.1% of that of the purified yeast catalase T (Table 1). Nonetheless, its catalatic activity was higher by an order of magnitude than that of the other haemoproteins which we have examined (Table 1). On starch gel zymograms, we found that 50 µg of this purified enzyme was sufficient to give an unmistakable catalase activity band. Fresh crude extracts of yeast always exhibit two catalase activity bands on starch gel zymograms, one fast moving and one slow moving band towards the anode (Figure 1, sample 3). Of the two catalase activity bands, the fast moving one was due to yeast catalase T, since it was removed from the zymogram when the crude extract was first mixed with an excess of antibody to catalase T (Figure 1, sample 4). This was confirmed by using pure antigen, in the presence and absence of antibody to catalase T (Figure 1, sample 1 and 2), where sample 1 contained purified catalase T

and sample 2 contained catalase T after treatment with antibody to catalase T. The slow moving activity band was found to have an identical Rf value to that of purified yeast cytochrome b_2 on the starch gel zymogram (Figure 1, sample 6). Pretreatment of cytochrome b_2 with antibody to catalase T did not remove its catalatic activity (Figure 1, sample 7), showing that the catalatic activity of the yeast cytochrome b_2 was not due to catalase T.

With aged crude extracts or extracts prepared from cells pretreated with n-butanol, three activity bands were observed. The third activity band moved between the fast and slow bands, slightly ahead of the slow moving band. This catalase activity band may be due to a proteolytic degradation (10) product of cytochrome b_2. We obtained identical results with crude extracts prepared from a variety of laboratory wild-type haploid and diploid strains of S. cerevisiae. Antibody to catalase T reacted specifically to its antigen and did not cross-react with cytochrome b_2. This was true in studies utilizing immunoprecipation coupled with enzyme assay, micro-immunodiffusion and the standard Ouchterlony procedure.

The zymogram did not detect the presence of catalase A or catalatic nucleoprotein despite their relatively high specific activities, presumably due to their low concentrations in the crude extracts.

In separate studies, pretreatment of purified yeast LDH with antibody to catalase A did not remove the slow moving catalase activity band on starch gel zymogram (data not shown). Amino triazole (AT) is a specific inhibitor of catalase (4). The catalatic activity of cytochrome b_2 was completely inhibited by 1×10^{-1} M AT while it had a little effect on the LDH activity (Figure 2). At this concentration of AT, yeast catalase was inhibited by 70%. With crystalline beef liver catalase, 50% inhibition was obtained with 5×10^{-4} M AT.

Weak as was its catalatic activity compared to that of catalase T, yeast cytochrome b_2 could be mistaken as a catalase isoenzyme on the starch gel zymogram. It is possible that similar non-catalase proteins which could catalyze the decomposition of H_2O_2 may also be present in crude extracts of organisms other than yeast; thus starch gel catalase zymograms of cell extracts should be interpreted with caution. In the absence of more conclusive data, catalase activity bands demonstrated by starch gel zymogram are, at best, descriptive, showing the presence of proteins which can catalyze the decomposition of H_2O_2 as opposed to a conclusive evidence for the presence of catalase(s) (28). For the same reason, it is equally misleading to assume that catalactic activity detectable by means of enzyme assay in a given crude extract preparation is due to the presence of catalase, particularly when the catalatic activity is low.

CONCLUSION

Precipitous readings of simple biochemical tests can frequently lead to erroneous conclusions. In this study, crude extracts of yeast were shown to exhibit two bands of apparent catalase activity on starch gel zymograms. One band was found to be due to catalase whereas the other was due to cytochrome b_2 activity. It is possible that similar non-catalase proteins, capable of catalyzing the decomposition of H_2O_2, could also be present in the crude extracts of organisms other than yeast. Therefore, starch gel catalase zymograms of such cell extracts should be always interpreted with caution.

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Figure 1

Starch gel zymogram showing catalase activity of purified yeast catalase T, crude extract of bakers' yeast and purified yeast cytochrome b_2. Slots 1, 3, 6 contained purified catalase T (4 µg), a crude extract of bakers' yeast (125 µg) and purified yeast cytochrome b_2 (700 µg), respectively. Slots 2, 4, 7 were as in 1, 3, 6, except that the preparations had been treated with excess antibody to catalase T, incubated at 37°C for ½ hr, centrifuged and the clear supernatant used for electrophoresis. Slot 5 contained tracking dye.

Figure 2

The effect of 3-amino-1,2-4-triazole on the catalase and LDH activities of purified yeast cytochrome b_2. Details in "Methods".

Figure 1

Starch gel zymogram showing catalase activity of purified yeast catalast T, crude extract of bakers' yeast and purified yeast cytochrome b_2. Slots 1, 3, 6 contained purified catalase T (4 μg), a crude extract of bakers' yeast (125 μg) and purified yeast cytochrome b_2 (700 μg), respectively. Slots 2, 4, 7 were as in 1, 3, 6, except that the preparations had been treated with excess antibody to catalase T, incubated at 37° C for 1/2 hr, centrifuged and the clear supernatant used for electrophoresis. Slot 5 contained tracking dye.

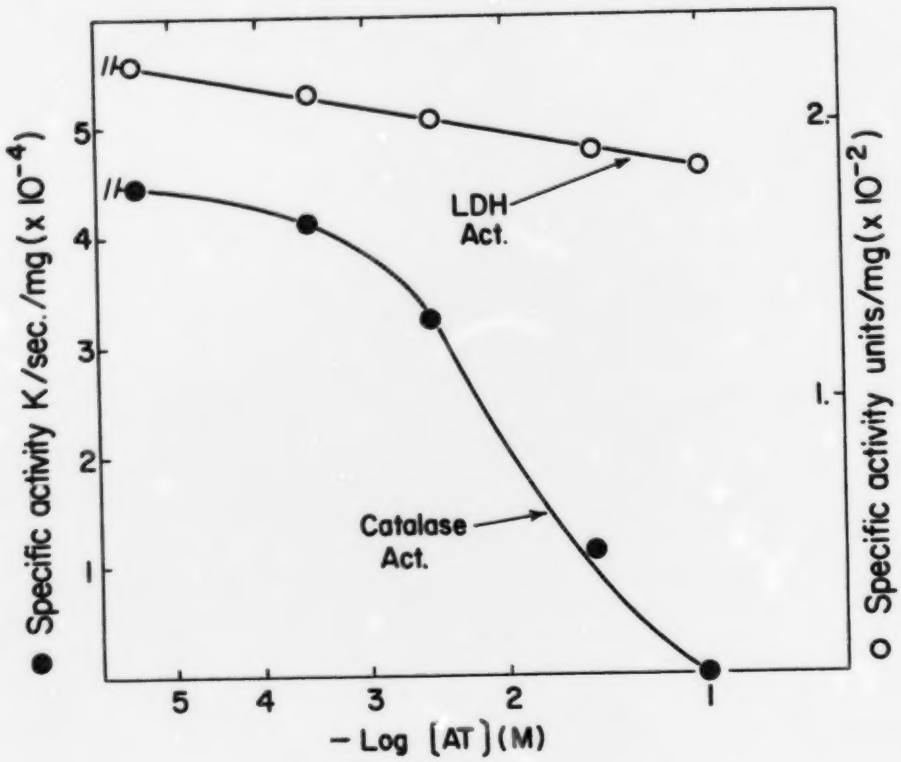

Figure 2

The effect of 3-amino-1,2-4-triazole on the catalase and LDH activities of purified yeast cytochrome b_2. Details in "Methods".

Table 1

Catalatic activity of non-catalase proteins

Enzymes	Specific activity K/sec/mg
Yeast cytochrome b_2	$4.90 \pm 1.39 \times 10^{-4}$
Haemoglobin (beef blood)	$4.00 \pm 1.20 \times 10^{-5}$
Cytochrome c	no detectable activity
Horse radish peroxidase	no detectable activity
Haemin (non-protein)	no detectable activity
Yeast catalase T	7.06×10^{-1}

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